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Rice leaf pathogenic fungi on wheat, oat, *Echinochloa phyllopogon* and *Phragmites australis*

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Summary. Pathogenic fungi that infect rice also infect a range of other plants. The mycoflora on a number of these plants in Morocco was studied. *Echinochloa phyllopogon* and *Phragmites australis* are two weeds adapted to rice fields. Wheat is often grown in rotation with rice, and common oat is an adventitious specie common in wheat fields. Fungi found in these plants were of two types: 1. True rice pathogens: *Pyricularia grisea, Helminthosporium oryzae, H. sativum, H. australiensis, H. spiciferum* and *Curvularia lunata* and 2. Saprophytes that cause rice discoloration: *Trichoderma harzianum, Alternaria alternata, Nigrospora oryzae, Epicoccum nigrum, Fusarium moniliforme, Cladosporium herbarum* and *Trichothecium roseum*. Seed discoloration also induces a weak germinative power of the paddy and lowers market value and yield at the manufacturing stage. Among these latter fungi, *T. harzianum*, *A. alternata* and *F. moniliforme* can be used to control foliar diseases caused by the true rice pathogens. This is the first report of *Helminthosporium oryzae* on wheat and oat in Morocco. The study also found that the pathogenic fungi *P. grisea*, *H. oryzae* and *H. sativum* isolated from wheat, oat, *Echinochloa phyllopogon* and *Phragmites australis* are strongly pathogenic when inoculated on rice.

Key words: rice, wheat, oat, *Echinochloa phyllopogon, Phragmites australis,* weeds, rice foliar diseases.

Introduction

Rice pathogenic fungi also attack many herbaceous species other than rice, but any rice pathogen isolated from a weed infects rice (Kato and Yamagushi, 1980). Ou (1985) listed all adventitious gramineous species that are sensitive to the rice pathogen *Pyricularia grisea* on the basis of natural infections or artificial inoculations on Gramineae in the field. Benkirane *et al*. (2000) showed by cross inoculation that Moroccan isolates of *P. grisea* from *Stenotaphrum secundatum* are also pathogenic to rice and that isolates from rice in turn attack *S. secundatum*.

Helminthosporium oryzae infects not only cultivated rice but also wild rice species such as *Zizania aquatica* and *Zizania palustris*. These are apparently the only other natural hosts of *H. oryzae* (Atkins, 1974) but many other Gramineae can become infected by artificial inoculation with this fungus: Nelson and Kline (1961) reported that *H. oryzae* caused foliar lesions on *Agrostis palustris, Alopecurus arundinaceus, Avena fatua, Avena sativa, Axonopus affinis, Bromus catharicus, Dactylis glomerata, Eleusine indica, Eragostis curvula, Festuca arundinacea, Festuca elatior, Festuca runa,*

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Hordeum vulgare, Lolium multiflorum, Lolium perenne, Oryza sativa, Panicum virgatum, Pennisetum spicatum, Phalaris tuberosa, Poa pratensis, Sorghum sudanense and *Triticum aestivum*. According to Ou (1985), artificially infected grass species other than rice are never or rarely invaded by *H. oryzae* under natural conditions.

In Morocco, the importance of crops grown in rotation, adventitious species and weeds in furnishing reservoirs of rice foliar pathogens has not yet been studied. Our objective was to determine the occurrence of rice leaf pathogens in: a rotation crop, wheat, a wheat adventitious plant, oat, a harmful weed of rice, *Echinochloa phyllopogon* and a species growing near rice fields, *Phragmites australis*. These last two species are not known to be host plants of rice foliar pathogens. The pathogenic power that those fungi found on adventitious species have against rice plants, and the degree to which different rice varieties are contaminated by those fungi will give an idea of their pathogenicity on rice.

Material and methods

Sampling method

Sampling of wheat and oat was done in Gharb wheat fields, where rice had previously been grown, between April 1 and May 30, 2001. Sampling of rice (*Oryza sativa* cv. Elio), *E. phyllopogon* and *Ph. australis* was done between August 30 and September 30, 2001, a period corresponding to the rice maturation stage. Sampling of wheat, oat, rice and *E. phyllopogon* was done diagonally according to Matsushima random technique (1966). Random sampling was done for *Ph. australis* found near rice fields. Plants were transported to the laboratory to identify the pathogens.

Isolating technique

Plants of rice, wheat, oat, *E. phyllopogon* and *Ph. australis* showing different kinds of lesions were analysed in the laboratory to detect the fungi associated with these lesions by a filter paper technique. Leaves with lesions were cut into small fragments, washed with water, disinfected in alcohol and placed in 90 mm Petri dishes on three filter paper discs moistened with sterile distilled water at the rate of 10 lesions per Petri dish. The lesions were incubated at 22°C alternating 12 h of darkness with 12 h of continuous light (white fluorescent tubes). Lesions were microscopically examined after 7 days and spores were taken with a capillary tube and placed on agar media to determine the fungi. Determination of species was done according to the Wang and Zabel manual (1990).

The contamination percentage $Cp = N_{FL} \times 100 / N_{TL}$ was calculated. N_{TL} is the number of total lesions, and N_{FL} the number of lesions contaminated by each fungus.

Inoculum preparation and inoculation technique

To complete the study, incubated leaf fragments were placed individually in tubes with 1 ml of distilled water and vortexed. The resulting spore suspension was filtered through muslin to remove the mycelium (Xiao *et al*., 1991). Spore concentration was adjusted with water containing 0.05% Tween and 0.5% gelatine to a final concentration of 10^5 spores ml^{-1}.

Six rice varieties were inoculated: Dinar, Bahja, 446, Kenz, Maghreb and Triomphe. Seeds were soaked in sodium hypochlorite (0.6%) for 10 min, thoroughly rinsed with sterile distilled water, dried on sterile filter paper and then pre-germinated in Petri dishes containing cotton soaked with sterile distilled water. Incubation was for 75 hours at 28°C in the dark. Plantlets were transplanted to jars and watered until the 3–4-leaf stage (4–5-week-old plants).

Young rice plants were inoculated by spraying with 60 ml of a spore suspension containing 10^5 spores ml^{-1} of *P. grisea*, *H. oryzae* and *H. sativum* isolated from leaf lesions of wheat, oat, *E. phyllopogon* and *Ph. australis*. Inoculated plants were kept in the laboratory for 48 hours under black plastic sheeting to maintain a relative humidity of about 100%, and were moved to the greenhouse thereafter.

Seven days after inoculation, the severity of infection on the rice leaf was scored according to Barrault's scale (1989) for *H. oryzae* and *H. sativum* and according to the scale of Notteghem *et al.* (1980) for *P. grisea*. The disease severity index (SI) was calculated: $SI\% = \Sigma X_i \times n_i / 9N_t$, where X_i is the disease severity score, n_i the number of plants with severity i, N_t the total number of plants, and 9 the highest mark on the scale.

Sporulation (spores/cm^2) of the identified pathogens on the infected rice leaves was estimated

according to the technique of Hill and Nelson (1983).

Statistical analysis

Results were tested for statistical significance using variance analysis and LSD test.

Results

Contamination percentage of leaf lesions by the different fungal species

Table 1 shows the principal fungi found on the leaf lesions of rice, wheat, oat, *E. phyllopogon* and *Ph. australis* with the contamination percentage of each pathogen on the plants studied. Fourteen species were identified on the plants, with varying contamination percentages. The same species were identified on rice, *E. phyllopogon* and *Ph. australis*.

Disease severity

Table 2 shows the severity indexes of *P. grisea*, *H. oryzae* and *H. sativum* from wheat, oat, *E. phyllopogon* and *Ph. australis* when inoculated from the six rice varieties. The SI values were high and included 20 for *P. grisea* from *Ph. australis* on rice variety 446, 76.7 for *P. grisea* from *E. phyllopogon*

Table 1. Contamination percentage (Cp in %) of leaf lesions on rice, wheat, oat, *Echinochloa phyllopogon* and *Phragmites australis* caused by the different fungi identified.

Fungus	Rice	Wheat	Oat	*E. phyllopogon*	*Ph. australis*
Pyricularia grisea	26	0	0	30	20
Helminthosporium oryzae	44	15	15	46	30
H. sativum	40	18	65	38	60
H. australiensis	38	0	0	30	40
H. spiciferum	14	0	0	24	70
Curvularia lunata	40	0	0	62	50
Alternaria alternata	90	83	97	100	100
Fusarium moniliforme	72	13	9	100	80
Epicoccum nigrum	50	18	75	64	10
Nigrospora oryzae	42	51	18	14	60
Trichothecium roseum	20	13	0	30	70
Cladosporium herbarum	50	24	32	18	54
Trichoderma harzianum	58	0	0	26	36
Penicillium sp.	0	6	9	0	0

Table 2. Severity indexes of *Pyricularia grisea*, *Helminthosporium oryzae* and *H. sativum* isolated from wheat, oat, *Echinochloa phyllopogon* and *Phragmites australis* on the leaves of six rice varieties. The severity of infection was scored seven days after inoculation, according to the scale of Notteghem *et al.* (1980) for *P. grisea* and according to Barrault's scale (1989) for *H. oryzae* and *H. sativum*.

Variety	*P. grisea*		*H. oryzae*				*H. sativum*	
	E. phyllopogon	*Ph. australis*	*E. phyllopogon*	*Ph. australis*	Wheat	Oat	Wheat	Oat
Dinar	23.3 c[a]	4.5 d	36.4 c	60.3 a	54.5 a	73.3 a	62.1 a	48.5 ab
Bahja	76.7 a	55.5 b	48.4 b	34.5 c	61.2 a	68.2 a	57.5 a	45.1 b
446	74.5 a	20.0 c	46.5 bc	36.0 c	30.5 b	43.5 b	35.5 b	63.3 a
Kenz	60.0 b	68.1 a	46.2 bc	45.2 b	58.1 a	65.3 a	60.5 a	41.6 b
Maghreb	58 0 b	50.1 b	68.3 a	44.2 bc	62.3 a	70.5 b	28.9 b	55.8 a
Triomphe	73.4 a	64.5 a	70.5 a	65.3 a	-	-	-	-

[a] Values with the same letters in the same column are not significantly different (LSD test). Results were tested for statistical significance using variance analysis and LSD test.

-, not determined.

Table 3. Sporulation (spores/cm^2) of *Pyricularia grisea*, *Helminthosporium oryzae* and *H. sativum* isolated from wheat, oat, *Echinochloa phyllopogon* and *Phragmites australis* on leaves of six rice varieties

Variety	*P. grisea*		*H. oryzae*				*H. sativum*	
	E. phyllopogon	*Ph. australis*	*E. phyllopogon*	*Ph. australis*	Wheat	Oat	Wheat	Oat
Dinar	2.1 c[a]	0.7 d	4.3 c	8.2 a	7.4 b	8.4 a	3.5 c	2.8 b
Bahja	7.4 a	5.1 bc	5.2 bc	4.5 bc	10.6 a	9.5 a	7.2 b	8.3 a
446	6.7 b	4.1 c	5.6 b	4.1 c	7.2 b	3.8 b	7.5 b	12.7 a
Kenz	6.5 b	7.0 a	4.9 c	5.1 bc	7.5 b	7.1 b	12.3 a	2.5 b
Maghreb	7.3 a	6.8 ab	8.3 a	5.3 b	6.1 b	2.7 b	4.5 c	2.1 b
Triomphe	6.8 ab	7.0 a	7.9 a	7.5 a	-	-	-	-

[a] Values with the same letters in the same column are not significantly different (LSD test)

on Bahja but only 4.50 for *P. grisea* from *Ph. australis* on Dinar. These values are comparable to the SI of *H. oryzae* from 209, Triomphe, Hayat, Arch and Elio rice varieties (Serghat, 2004) (between 24.6 and 87.4).

Sporulation of pathogens isolated from wheat, oat, *E. phyllopogon* and *Ph. australis* on rice leaves

Pyricularia grisea, *H. oryzae* and *H. sativum* sporulated on all rice varieties tested (Table 3). Nevertheless, rice varieties must not be termed sensitive or resistant, because the behaviour of each variety depended on the pathogen and its origin. Sporulation values varied from 0.7 spore/cm^2 for *P. grisea* from *Ph. australis* on Dinar, to 12.7 spores/cm^2 for *H. sativum* from oat on 446.

Discussion and conclusion

Helminthosporium oryzae, identified on wheat and oat with a contamination percentage of 15% is a rice pathogen. This is the first time in Morocco that this pathogen has been isolated from wheat and oats, on which it induces the same lesions as on rice. On the other hand, *H. sativum* is a common wheat pathogen that attacks the roots (Lyamani, 1988), but also the leaves (Rieuf and Teasca, 1973). This pathogen has been isolated from rice and its pathogenicity has been examined (Ouazzani Touhami *et al*., 2000).

That some rice pathogens also infect wheat and oat was also reported by Ou (1972) and Vidhyasekaran (1986) who examined rice pathogens on corn and wheat, and by Nelson and Kline (1961) who showed by artificial inoculation that *H. oryzae* induces leaf lesions on Gramineae such as corn, wheat, oat and sorghum. This suggests that *H. oryzae* maintains its activity outside the rice vegetative cycle and survives on wheat as a rotation or neighbouring crop, and on oat, a rice adventitious species, where it induces the same lesions as on rice. Wheat matures precisely at the time when the rice crop starts growing, so *H. oryzae* can resume its activity on rice as soon as this crop starts growing.

Fourteen fungal species were identified on rice, wheat, oat, *E. phyllopogon* and *Ph. australis*. They can be divided into two groups.

a) Rice pathogens: *P. grisea* (Benkirane *et al*., 2000); *H. oryzae* (Bouslim *et al*., 1997), *H. spiciferum* (Ennafah *et al*., 1999), *H. sativum* and *H. australiensis* (Ouazzani *et al*., 2000) and *Curvularia lunata* (Hassikou *et al*., 1997).

b) Saprotrophes: *Trichoderma harzianum*, *Alternaria alternata*, *Fusarium moniliforme, Nigrospora oryzae*, *Epicoccum nigrum*, *Cladosporium herbarum* and *Thrichothecium roseum*. These fungi, though they do not cause serious damage on the aerial parts of plants, are seed discoloration agents (Gnancadja, 2002). Seed discoloration reduces the germinative power of the paddy and lowers market value and yield at the manufacturing stage (Jin *et al*., 1994).

Trichoderma harzianum, *A. alternata* and *F. moniliforme* found on the leaf lesions of rice, wheat, oat, *Ph. australis* and *E. phyllopogon* have a potential use in protecting rice leaves against the serious damage caused by true pathogens: *P. grisea*, *H. oryzae*, *H. sativum*, *H. australiensis*, *H. spiciferum* and *C. lunata* (Ouazzani Touhami, 2001).

The introduction of *T. harzianum* to the rice plant phyllosphere significantly reduces rice blast caused by *P. grisea* (Ouazzani Touhami *et al.*, 1997) and brown spot disease caused by *H. oryzae* (Mouria *et al.*, 1997). The protection consists in a reduction of foliar symptoms and of the sporulation capacity of the pathogens on the leaves.

Pathogenic fungi on the foliar lesions of wheat, oat, *Ph. australis* and *E. phyllopogon* are a potential source of inoculum for rice plants. The presence of these plants in and around rice fields maintains high levels of contaminant inoculum. The multiplication of sporulating lesions on wheat and weeds causes new infections and accelerates the spread of epidemics on the rice crops, particularly during the first cycles of an epidemic.

According to Boulet and Bouhache (1990), the presence of adventitious plants adapted to rice growing conditions, such as *Echinochloa* spp. *(E. crus-galli* and *E. phyllopogon*), is a serious hazard to the health of rice fields. These plants harbour the same fungi as are found in rice leaf lesions. El Abdellaoui (2001) showed that *P. grisea* isolates from *E. crus-galli* and *O. sativa* have the same sexual compatibility sign and are equally pathogenic to rice.

Rice field adventitious plants can also serve as hosts for other enemies of rice: viruses, bacteria and insects. According to Bouhache *et al.* (1989), the weeding of rice fields and the eradication of adventitious plants probably protects rice plants from infection by the spores that develop on those adventitious plants. Wheat, oat, *E. phyllopogon* and *Ph. australis* are here first reported as host plants of rice pathogens.

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